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From Captivity to Conservation Success: A Review on the Mouse Deer Breeding Program and its Implications for Biodiversity Preservation

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Abstract

Captive breeding has become an important tool for conserving threatened species. The success of these conservation programs depends on the survival of species through self-sustaining populations managed by scientific values. Mouse deer is a primitive deer that plays a crucial role in the forest ecosystem as a key seed disperser and forms significant prey for both small and large predators. Despite its significance, little is known about this species' mating behavior and reproductive physiology in both the wild and captivity. As part of the conservation breeding and species recovery program, a breeding program of mouse deer started with the aim of breeding them in captivity and release them into the wild to preserve the biodiversity. This program began with six founder individuals and we observed a remarkable increase of 400 individuals within 10 years. These captive-bred individuals have been successfully introduced into the wild. This paper presents a comprehensive review of potential factors required for the successful breeding program and also provides recommendations on future directions and perspectives of conservation breeding program of mouse deer and other species.

Keywords: Captive Breeding, Conservation, Ex-Situ, Mouse Deer, Reintroduction

1. Introduction

Biodiversity is essential for maintaining ecological balance and stability. Each species plays a unique role in the ecosystem, and losing a species can have a great impact on the entire ecosystem. In particular, mammals play an essential role in ecosystems as prey, predators, seed dispersers, etc. The survival of many plant species depends on animals for pollination and seed disperser activities, which help to regenerate and maintain forests. However, many mammals are at risk of extinction and facing severe threats because of fragmentation of forests, habitat loss and degradation, poaching and hunting, pollution, climate change and anthropogenic disturbances, etc. To protect these species from extinction, a wide range of conservation measures are necessary for the protection and increase their populations, which include the "*in-situ*" conservation method, whereby a species can be conserved in their natural environment while "*ex-situ*" conservation method refers to the conservation of biodiversity outside its natural habitat. The *ex-situ* approach involves bringing the plant or animal species from their natural environment and placing them in a controlled environment, such as a zoo, botanical garden, or breeding center, where they can be protected, bred, and managed. The captive breeding is also one such method to increase their numbers and release them into wild.

Conservation breeding programs have become a widespread and important tool to save the endangered species due to the loss of natural habitat and populations. Conservation breeding helps in maintaining genetic diversity, sustainable population, and fitness. The captive breeding program's success depends on the survival of species through self-sustaining populations managed by scientific principles. These programs use a pedigree-based mean kinship strategy[1] to check the unrelatedness

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of the founder population. However, captive breeding is expensive and requires a lot of scientific managements. The captive breeding program faces two fundamental problems; the first one is the management of several generations' population in captivity without the loss of genetic variability, and the second is the non-availability of enough space in zoo to keep all taxa in captivity[2]. Without reintroducing captive bred population in their natural habitat and establishing a viable population following introduction, conservation breeding is a failure. The success of conservation breeding revolves around multidisciplinary approaches that involve science, habitat preservation and habitat restoration, species survival, genetic diversity, demographic data, environmental education, policies, and interactive management.

Mouse deer (*Moschiola indica*) belonging to the Cetartiodactyla order and family of ruminants called Tragulidae. They are the world's smallest hoofed animals. Mouse deer is listed as "least concern" status in the International Union for Conservation of Nature (IUCN) Red List. However, because of poaching and hunting in the wild by local tribes for their bushmeat, it is recognised as schedule-l species (endangered) in the Indian Wildlife Protection Act 1972. Mouse deer are nocturnal and elusive with three-chambered stomach, unlike four chambers in other deer[3]. Of the three chambers, the rumen is the largest chamber for storage of food, while the other chambers are the reticulum, where microorganisms ferment the food, and the abomasum for digestion of food using gastric juice in lesser mouse deer[4]. However, mouse deer lack a true omasum stomach chamber, which is usually present in the other deer species that are called four-chambered stomach ruminants[5]. Both sexes have large upper canines, but they are more prominent in males than females. Males use their elongated canines as an effective weapon in fights with other males over females for mate selection. They are mainly herbivores and their diet primarily comprises fallen fruits, seeds, shoots, flowers, leaves, bushes etc[6]. Mouse deer are widely distributed across the Indian subcontinent, ranging from the tropical moist deciduous forests to the evergreen forests of peninsular India. Mouse deer have been recorded in the Western and Eastern Ghats, Central India, and the Terai region of the Gangetic plains[7]. However, because of poor sighting records, the complete distribution of mouse deer is unknown.

Despite their widespread distribution, mouse deer populations are decreasing because of rapid habitat destruction and forest fragmentation, particularly in disturbed forest areas. In addition, hunting pressure by indigenous tribal communities is believed to be a major factor in the species' population reduction in the Western and Eastern Ghats, although other factors, such as habitat destruction and forest fragmentation, also play a role[8]. As a result, the low population density of mouse deer makes them an extremely vulnerable species with a high risk of extinction. No information on the reproductive physiology of mouse deer is available, including the length of the oestrus cycle and gestation, pregnancy, and age at sexual maturity. Therefore, Nehru Zoological Park, Hyderabad, initiated a mouse deer conservation breeding program in 2010 in association with the Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology, supported by Central Zoo Authority, Government of India, having 6 individuals as founder stock (2 males and 4 females). This program aimed to breed the mouse deer in captivity and release them back into the wild. In this paper, we briefly discuss the general strategies and factors required for a successful conservation breeding program of mouse deer, and some recommendations for the future are presented for effective captive breeding in India or elsewhere.

2. Breeding Program and Reintroduction

The success of a reintroduction program from a captive-bred population depends on several scientific factors. These include selecting a founder stock of genetically unrelated individuals, scientific involvement, and having a dedicated facility. An example of a successful conservation breeding program and reintroduction is the mouse deer program, which has brought the species back from the local extinction (Figure 1).

This program began in 2010 with a founder population of six individuals with active collaboration between Nehru Zoological Park, Hyderabad, LaCONES, CSIR-CCMB, and the Central Zoo Authority of India. With a successful breeding the mouse deer population has reached a maximum of 232 individuals by March 2019 (Figure 2). Following successful breeding, the first batch of eight captive-bred mouse deer was released

Figure 1. Mouse deer (*Moschiola indica*) in conservation breeding facility at Nehru Zoological Park, Hyderabad.

into the Mannanur Range of Amrabad Tiger Reserve, Telangana, in July 2018. Since then, eight batches, comprising 72 individuals, have been released into the wild in the Amrabad Tiger Reserve and Kinnerasani Wildlife Sanctuary. Currently, the mouse deer population has reached approximately 400 individuals, with half of them successfully reintroduced into the forests of Pocharam Wildlife Sanctuary, Mrugavani National Park, Jannaram Forest, and Nirmal Forest. Since captive-bred populations cannot be released directly into the wild, a soft release facility was established in the forest (Figure 3). The facility uses a three-stage process for conditioning the mouse deer. The first stage is stabilization, which lasts at least 14 days and involves feeding the mouse deer processed fruits, as in a conservation breeding facility. The percentage of processed fruits gradually decreases, and for the last four days no processed fruits are given, encouraging the mouse deer to forage for native fruits and leaves in the surrounding forest. The second stage is acclimatization, which lasts 14 days and involves feeding the mouse deer with natural fruits and fallen leaves while foraging. The last stage is the pre-release stage, where there is no time limit, and the door is kept open for the mouse deer to voluntarily move into the wild. CCTV cameras are installed in all compartments for surveillance and recording of behavior. Post-release monitoring of the mouse deer is done using camera traps, and each mouse deer is tagged with a transponder with a unique ID for individual identification. Regular health check-ups are performed by physically restraining the mouse deer.

3. What Made a Successful Captive Breeding Program?

3.1 Reproductive Monitoring of Captive Population

Endocrine monitoring of reproduction is crucial to maintain a sustainable population and reproductive success in the captive breeding of threatened animals. It plays an important role in enhancing the success of natural breeding and assisted reproductive technologies and allows an understanding of species biology. However, information on the reproductive physiology of animals is limited, such as length of the estrus cycle, gestation length, seasonality, and age of puberty. Non-invasive reproduction monitoring is a well-established technique to study the reproductive physiology across mammals[9-11]. In captive breeding of mouse deer, fecal pellets were collected to measure the reproductive hormones, including progestogens, estrogens, and testosterone, to study the seasonality, pregnancy diagnosis, post-partum estrus, age of sexual maturity, etc[12]. This information contributed to planning a successful breeding pair, a suitable time for mating, reproductive seasonality, and estrus synchronization in captive breeding of mouse deer.

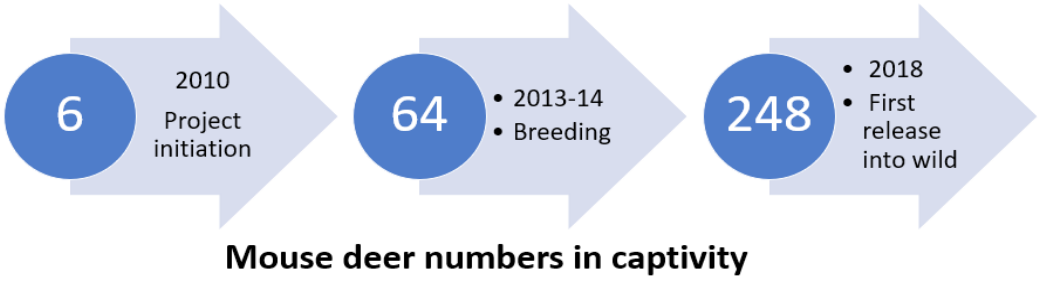

Figure 2. Milestones of the mouse deer captive breeding program at Nehru Zoological Park, Hyderabad.

Furthermore, we discovered a unique post-partum estrus in mouse deer whereby mating occurred within 4-6 hrs following the delivery of fawn[3]. The occurrence of post-partum estrus helped us to strategically plan mating by introducing a fertile male into the enclosure during the last stage of pregnancy to enhance the reproductive output.

3.2 Genetic Studies and Demography Management of Captive Population

One of the key factors for a successful captive breeding program is to have a genetically healthy population and demography management of the captive population. It is a crucial aspect in maintaining a sustainable population as it mitigates the genetic challenges, which include loss of genetic diversity due to genetic drift and deleterious effects of inbreeding depression. Therefore, genotyping of the captive-bred population is required to measure the genetic heterozygosity of the captive inbred or outbred population. It is essential to ensure that enough number of genetic unrelated breeding pairs are available with a balanced sex and avoid aging individuals for mating prior to start any captive breeding program. For example, while initiating a captive breeding program of mouse deer, the founder stocks (two males and four females) were from different forest locations and supplemented with captive-born individuals from various zoos. An assessment of inbred individuals, if any, in the captive populations is required using microsatellite molecular markers. Further, it needs to be done prior to the reintroduction to the wild. The major goal of a captive breeding program is to maintain 90% of the genetic diversity of the source population for 100 years[13]. However, the specific time frame varies depending on the species and goal of each program. For example: in black-footed ferret (*Mustela nigripes*) and Guam rail (*Rallus owstoni*), conservation breeding program aimed to preserve 90% of the genetic diversity for 50 years due to short generation time for these species and the intention to rapidly establish of multiple wild populations. Overall, genetic profiling plays a critical role in the success of captive breeding programs, particularly for endangered species like mouse deer. Ensuring a genetically outbred population improves the chances of survival and success following reintroduction into the wild[13].

3.3 Habitat Restoration and Reintroduction

The fundamental goal of each captive breeding program is to release the species back into the wild. However, not all species are able to be reintroduced into the wild due to habitat degradation and loss. Before reintroducing the species, one can checklist the components of the successful reintroduction program. These components include habitat preservation and management, such as habitat protection from degradation and exploitation, restoration and management of degraded habitats, and enhance the number of preservation areas[13]. A species cannot be reintroduced into their native habitat until the leading cause of species decline would be identified and eradicated or reduced. In addition, the reintroduced

Figure 3. Mouse deer are being released into the wild at Pocharam Wildlife Sanctuary in Medak district in June, 2021.

habitat should be legally protected from illegal poaching or hunting activities. Mouse deer have been reintroduced into the protected areas in the national park and wildlife sanctuaries where maximum protection is available from hunting and poaching. Captive bred individuals often show high mortality rates when released into the wild due to domesticated or inappropriate behavior[14]. For example, these individuals find difficulty in foraging for food in the wild or are unable to avoid predators. Therefore, a soft release facility was established in the forest for mouse deer to avoid these difficulties. Mouse deer were monitored for foraging behavior and natural diet in the pre-release stage before releasing into the wild.

3.4 Scientific Participation in Conservation Breeding Programs

Understanding reproductive biology is essential for successful captive breeding and reintroduction programs. During the mouse deer breeding program, we unravelled the reproductive physiology, reproductive behavior, reproductive characteristics like gestation, estrus, etc., and genetics regarding inbreeding. Some key points highlighting the importance of scientific involvement are given below.

3.4.1 Behavioral Studies

Studies on reproductive performance and breeding behavior are important factors that provide insights into the reproductive traits of animals, such as mating behavior, gestation length, estrus cycle, etc. These characteristics can be controlled by various factors such as age, genetics, diet, environment, and management techniques. Mate selection and mating behavior both impact the likelihood of successful copulation, fertilization, the genetic makeup of the offspring, and their chances of survival[15]. Hence, it is essential to study breeding characteristics such as reproductive behavior, mating systems, reproductive anatomy and hormonal regulation, in order to understand the reproductive biology of animals. Breeding characteristics can vary widely between species and even the sex of the same species, which have to be considered during the development and implementation of conservation breeding program. This knowledge would effectively help strategize optimal breeding schemes to increase breeding success, maintaining genetic diversity and consequently boosting population size in captivity.

3.4.2 Endocrine Monitoring

Understanding of reproductive physiology is essential to monitor the endocrine function and reproductive status. A complex system of hormones, physiology, nutrition, environment, etc., regulates the reproductive process in animals. Hormones, secreted by the endocrine glands from specific organs of the body, are fundamental chemicals that regulate the physiology and behavior of vertebrates. Monitoring of reproductive hormones provides crucial information such as timing of ovulation, estrus cycle, fertility status, gestation length, etc. By monitoring hormonal changes, one can determine the optimal time for breeding and can use hormone treatments to stimulate reproduction in captive breeding programs.

3.4.3 Dedicated Team with Long-term Vision

For a successful breeding program, a dedicated and exclusive team that comprises trained veterinarians, research biologist and zoo keepers is required. Here, we had an exclusive team that looks after everything from feeding and health care to maintenance of various breeding enclosures. Further, scientists, conservationists, zoo managers, and policymakers should work in harmony to achieve a successful breeding program.

3.4.4 Public Awareness and Education

Raising public awareness and education programs about endangered species and the importance of conservation efforts are crucial for an effective conservation programme. Scientists may help to educate the public, policymakers, and communities by addressing the significance of conservation breeding programmes and the role of these efforts to preserve biodiversity and ecosystem.

4. Future Perspective

4.1 Studying the Nutritional Diet during Post-partum Estrus, Mating, and Pregnancy of Mouse Deer

In our study of breeding characteristics of mouse deer, we found that mouse deer breed throughout a year and

do not have a particular breeding season. In most of cases, females conceive following the mating and become pregnant, and this cycle continues throughout their life span, provided the mating is successful. Future research should involve understanding female mouse deer's energy and nutritional intake during reproductive cycle, pregnancy, and lactational time as mouse deer continue to be pregnant most of their lifetime. Overall, it would be interesting to study how mouse deer balance their nutritional intake and energy demands during post-partum estrus and mating.

4.2 Monitoring of Diseases and Parasite Prevalence in Captive Mouse deer

The population of mouse deer in the conservation-breeding centre can be at risk of pathogenic infections due to their proximity to other animal enclosures in the zoo. Therefore, it is essential to screen captive individuals for common pathogens that affect mouse deer before being released into the wild, as well as pathogens that affect other animals such as ungulates in the zoo. A number of diseases, such as pneumonia and leptospirosis, have been reported in captive-bred mouse deer and other ungulates of the zoos during the last five years**.** Moreover, tuberculosis is endemic in several Indian zoos, and this is a potential concern for the population of mouse deer in the conservation-breeding centre. Therefore, regular monitoring is crucial for the population of captive mouse deer. Gastrointestinal parasites should also be monitored, and individuals in which parasites are found should not be selected for the release. To ensure that the mouse deer population is free of diseases and parasites, regular screening should be conducted during the conditioning process. This can be achieved using molecular markers and microscopic analysis of randomly collected fecal pellets.

Overall, the conservation-breeding centre facility must take significant precautions to ensure that the mouse deer population is healthy and disease-free before being released. This involves regular monitoring and screening of diseases and parasites, as well as proper communication with the release facility to prevent the spread of diseases. By taking these measures, the conservation-breeding facility can help to conserve the mouse deer population while minimizing the risks of pathogenic infections.

5. Problems and Challenges in the Reintroduction of Captive Breeding Animals to Wild

Modern zoos play an important role in education, awareness, wildlife conservation, and research through ex-situ programs. In recent years, captive breeding program gained more attention in the self-sustaining populations and recovery of threatened species. However, not all endangered species breed in captivity, self-sustaining, and successfully reintroduced into the wild. For example, the endangered oribi (*Ourebia ourebi*) was bred in captive breeding facility and reintroduced to their suitable habitat. Within two months of reintroduction, seven out of ten oribi were dead because of predation by humans and natural predators[16]. Another example is the reproductive failure of southern white rhinoceros (*Ceratotherium simum simum*) in a captive breeding program contributed to a severe population crisis for these species. The white rhino's reproductive failures show an inability to produce viable offspring in captivity due to the dominant behavior of founder females, which suppressed reproduction in first generation females[17].

Conservation breeding program should be aware of two fundamental factors: genetic diversity and minimizing mean kinship. These two factors include the preservation of genetic diversity and keep reproductive fitness by minimizing the random genetic drift across the pedigree population. In addition, other factors should also be considered, such as disease outbreaks, high cost or financial resources to maintain captive breeding programs, domestication behavior of species, poor success in reintroductions, logistics issues including transfer of animals, space availability and diet arrangement, reproductive age and overall health of the animal. However, these recommendations are not applicable across all the species and taxa and the requirements could be species-specific.

6. Conclusion

In this paper, we have reviewed the potential fundamentals required for a successful captive breeding programme using mouse deer as a model species. We recommend future perspectives on mouse deer captive breeding and reintroduction into the wild.

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